Bacteriostasic activity of Anthocyanin of *Malva sylvestris*

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Abstract: Bacteriostasic activity experiments on anthocyanin extracted from *Malva sylvestris* inhibiting *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, were conducted by using solid and liquid culture methods. The results showed that the anthocyanin of *M. sylvestris* had a great bacteriostasic activity to *Staphylococcus aureus* but had no bacteriostasic activity to both *Escherichia coli* and *Aspergillus niger*. The bacteriostasic activity to *Staphylococcus aureus* increased with increasing content of anthocyanin of *Malva Sylvestris* in the solid-culture experiment. The average diameters of bacteriostasic circle for *Staphylococcus aureus* was 6, 13.5, and 16.0 mm at 10g·L^{-1}, 20g·L^{-1}, and 30g·L^{-1} contents of anthocyanin of *M. sylvestris*, respectively. Moreover, this bacteriostasic activity kept long time as anthocyanin was of the high concentration.

Keywords: *Malva Sylvestris*; Anthocyanin; Bacteriostasic activity; Function
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Introduction

Malva sylvestris belongs to Malva, Malvaceae and contains much anthocyanin that is a kind of very important natural and functional pigment (WANG *et al.* 2003). The experiments on anthocyanin extracted from *Malva sylvestris* inhibiting *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* was carried out. *Aspergillus niger* plays an important role in food production, but can cause food putrefaction and deterioration or produce toxin to harm the people's health. *Staphylococcus aureus* can produce exogenous toxin and enterotoxin. These bacteria have a close relation with human activity for its broad distribution on the earth. They have the important effects on brewage, fermentation, antibiotic and enzyme industry. This study is helpful to find a new way to inhibit and kill deleterious bacteria.

Materials and methods

Anthocyanin of *Malva sylvestris* was homemade. *Staphylococcus aureus* 25923, *Escherichia coli* 25922 and *Aspergillus niger* were supplied by Heilongjiang Institute of Microbiology. Mildew culture medium was composed of 2-g $NaNO_3$, 1-g K_2HPO_4, 0.5-g KCl, 0.5-g $MgSO_4$, 0.01-g $FeSO_4$, 30-g Sucrose, 1000 mL Distilled water and 15-g agar.

Preparation of bacteria culture medium (Wang 1998)
The 10-g Peptone, 3-g beef extract, 5-g NaCl and agar of 15–20g were prepared in advance. These ingredients except of agar dissolved in 1000-mL distilled water and were added about 2 mL of 15% NaOH to adjust pH value to 7.2–7.4. Then agar was added and boiled to melt (adding no agar when adopting liquid culture).

Determining thalli growth by absorbency value (You-Jin Jeon *et al*. 2001)
Liquid nutrient culture medium of 50 mL was decanted to a triangular flask as a check sample. Three dosage-groups were designed, each group with liquid nutrient culture medium of 40 mL, and repeated 10 times. Then 10 mL of 10 g·L^{-1}, 20g·L^{-1}, and 30-g·L^{-1} anthocyanin sample after filtering through G3 filter were added to the medium in the triangular flask, respectively. The three groups were marked as #1, #2, #3, and the check-group was marked as #0. The experiments of inhibiting *Staphylococcus aureus* and *Escherichia coli* were conduted as follows: 0.1 mL of mother thalli solution was put in each triangular flask by pipette (the identical culture medium without adding thalli solution as reference solution respectively), cultured at 37 °C for 48h, and measured the O.D. value of these solutions (#0, #1, #2, #3) at 640 nm every 4 h.

Determination of thalli growth by speed-rate method (Gong 2005)
Under the sterile condition, 0.1 mL of *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* were separately mixed with 3.5 mL of culture medium, and then were uniformly spread on the bottom of three culture plates. Sterilized absorption filter papers, with 6-mm diameter for each one, were soaked separately in distilled water, 10- g·L^{-1} sample solution, 20- g·L^{-1} sample solution, 30- g·L^{-1} sample solution for 30 min. Then these filter papers were put symmetrically on the culture plate in cross shape. Site 1 was distilled water, site 2 was 10- g·L^{-1} sample solution, site 3 was 20- g·L^{-1} sample solution and site 4 was 30- g·L^{-1} sample solution. Four chips were put at each site and culture plates were set in the incubator. The bacteria were cultured at 37°C for 72 h, and the mycete at 28°C for 36h. Finally, the diameter of bacteriostasic circle was measured by cross-method.

Results

Bacteriostasic activity of Anthocyanin of *M. sylvestris* to *Staphylococcus aureus*
The result (Table 1) showed that O.D. value in 10- g·L^{-1} dosage-group increased from 0.108 to 2.352 after 48h while O.D. value of dummy increased from 0.114 to 1.623. Thus, the rising

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amplitude in 10- g·L^{-1} dosage-group was higher than that of dummy. When the content of anthocyanin was 20 g·L^{-1} and 30 g·L^{-1}, the corresponding O.D. value increased from 0.112 and 0.165 to 0.964 and 0.35, respectively, whose rising amplitudes were lower than that of dummy by far. It suggested that the low content of anthocyanin of *M. sylvestris* could accelerate the growth of *Staphylococcus aureus*, and with increasing of its content, its bacteriostasic activity to *Staphylococcus aureus* would be improved.

Table 1. Effect of anthocyanin in different content on the O.D. value of *Staphylococcus aureus* in liquid culture medium for 48h

Content	0 h	4 h	8 h	12 h	24 h	36 h	48 h
dummy	0.114	0.235	0.329	0.506	0.653	1.003	1.623
10 g·L^{-1}	0.108	0.228	0.375	0.429	0.677	1.583	2.352
20 g·L^{-1}	0.112	0.124	0.137	0.228	0.329	0.635	0.964
30 g·L^{-1}	0.165	0.173	0.184	0.193	0.22	0.27	0.35

Furthermore, the test of significance difference (Table 2) showed that all the three contents of anthocyanin of *M. sylvestris* (10 g·L^{-1}, 20 g·L^{-1} and 30 g·L^{-1}) had great significant effect on *Staphylococcus aureus* (t=30.36>$t_{0.01\ (9)}$=3.250). Of them, 30 g·L^{-1} of anthocyanin of *M. sylvestris* had the most favorable bacteriostasic activity.

Table 2. Effect of anthocyanin of *M. sylvestris* with different contents on bacteriostasic activity to *Staphylococcus aureus*

Dosage-group	Standard deviation(s)	Freedom (d*f*)	*t*	$t_{0.01\ (9)}$
10 g·L^{-1}	0.0235	9	31.02**	3.250
20 g·L^{-1}	0.041	9	15.80**	
30 g·L^{-1}	0.0365	9	32.87**	

Bacteriostasic activity of Anthocyanin of *Malva sylvestris* to *Escherichia coli*

The O.D. value of *Escherichia coli* in liquid culture medium within 48h is shown in Table 3. Just after adding *Escherichia coli*, the initial O.D. value of dummy, 10- g·L^{-1} dosage-group, 20-g·L^{-1} dosage-group and 30-g·L^{-1} dosage-group were 0.138, 0.141, 0.148 and 0.156, respectively. After 8 h, the values turned into 0.501 (dummy), 0.505 (10-g·L^{-1} dosage-group), 0.576 (20-g·L^{-1} dosage-group) and 0.662 (30-g·L^{-1} dosage-group), respectively, and all groups showed no inhibitory action and *Escherichia coli* presented the nature-growing trend, and 24 hours later, the O.D. values increased to 0.843 (dummy), 0.846 (10- g·L^{-1} dosage-group), 0.876 (20- g·L^{-1} dosage-group) and 1.000 (30-g·L^{-1}dosage-group), respectively, all the group still showed nature growth. The values of each group reached a peak (2.5) at 36 h. The curve of inhibiting *Escherichia coli* is almost one curve (Fig. 1) and not as trenchant as that of inhibiting *Staphylococcus aureus*. By the result it was easy to find that the content range of anthocyanin of *M. sylvestris,* 10–30·L^{-1}, had no inhibitory action to *Escherichia coli* within 48 hours. *Escherichia coli* showed the nature-growing trend in mixed culture medium with different content of anthocyanin and had no obvious difference from the dummy.

Table 3. The effect of anthocyanin of *M. sylvestris* in different content on the O.D. value of *Escherichia coli* in liquid culture medium within 48h

Content	0 h	4 h	8 h	12 h	24 h	36 h	48 h
Dummy	0.138	0.295	0.501	0.54	0.843	2.5	2.5
10 g·L^{-1}	0.141	0.312	0.505	0.558	0.846	2.5	2.5
20 g·L^{-1}	0.148	0.336	0.576	0.596	0.876	2.5	2.5
30 g·L^{-1}	0.156	0.367	0.662	0.683	1.0	2.5	2.5

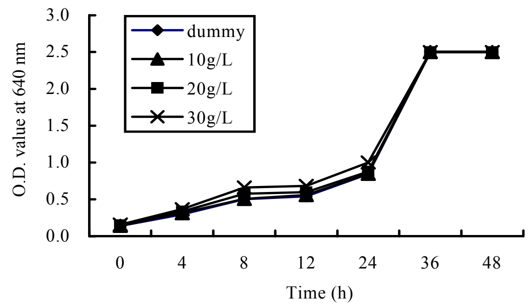

Fig.1 The curves of inhibiting *Escherichia coli* in liquid medium containing anthocyanin

Determining bacteriostasic activity by speed rate of thalli growth

The absorption filter papers soaked in different contents of anthocyanin of *M. sylvestris* were put on the surface of culture medium with subjected cultures. After culture, the diameter of bacteriostasic circle was measured. It was found that anthocyanin of *M. sylvestris* had a significant inhibitory action to *Staphylococcus aureus*, but had no obvious inhibitory action to *Escherichia coli,* and *Aspergillus niger* with no bacteriostasic circle formed around filter-papers (Fig. 2, 3 and Table 4).

Table 4. Relationship between diameter of bacteriostasic circle and content of anthocyanin (mm)

Content (g•L^{-1})	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillus niger*
10	6.0	—	—
20	13.5	—	—
30	16.0	—	—

When the content of anthocyanin of *M. sylvestris* was 10 g·L^{-1}, the diameter of bacteriostasic circle was 6 mm. With the content of anthocyanin increasing, the diameter of bacteriostasic circle increased (13.5 mm for 20 g·L^{-1}, 16.0 mm for 30g·L^{-1}), (Table 4), while inhibition ratio became 28.0% and 34.0%, respectively. It was suggested that the higher the content of anthocyanin was, the more obvious the effect of bacteriostasic on *Staphylococcus aureus* would be.

It was concluded that anthocyanin of *M. sylvestris* could inhibit *Staphylococcus aureus* effectively. *Staphylococcus aureus* has very strong resistance to heat, and it can not be killed during the food processing by boiling, pasteurizing, cooking and other heat treatments. Thus, adding anthocyanin should be a significantly available measures to prevent food from being polluted by *Staphylococcus aureus*.

Fig. 2 Bacteriostasic circles formed around anthocyanin of *M. sylvestris* in solid medium spreading *Staphylococcus auretus*

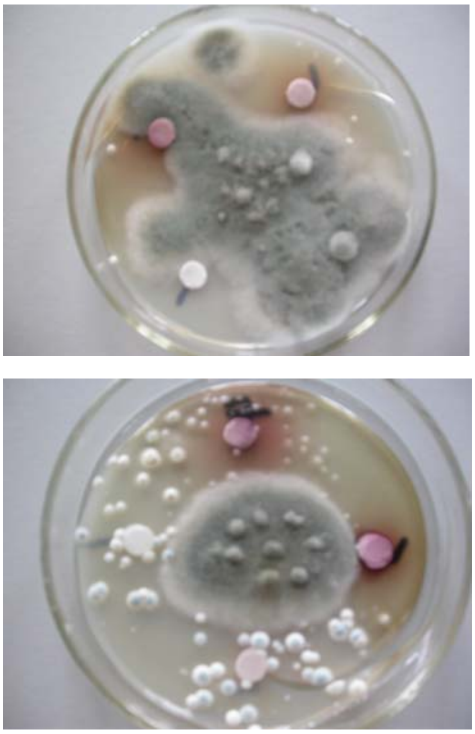

Fig. 3 No bacteriostasic circle formed around anthocyanin of *M. sylvestris* in solid medium spreading *Aspergillus niger*

Discussion

This study demonstrated that anthocyanin of *M. sylvestris* had a significant bacteriostasic activity to *Staphylococcus aureus*. This result is first time to be reported in China and abroad. *Staphylococcus aureus* can produce exogenous toxin and enterotoxin, which can infect skin injure of humans and animals to cause pyogenic symptom. When people eat the food polluted by *Staphylococcus aureus*, the toxin-type food poisoning will be induced. When *Staphylococcus aureus* gets into body along with food, it will be absorbed into blood in digestive tract and toxin having produced by the bacteria will stimulate central nervous system to lead to toxic reaction. *Staphylococcus aureus* has a very strong resistance to unfavorable factors outside and it even is the strongest bacteria of no-producing brood cell (Jia *et al.* 2001). It can be killed at 80℃ for 30 min or 1h. Anthocyanin of *M. sylvestris* has the great bacteriostasic activity to *Staphylococcus aureus*. This is probably due to the fact that anthocyanin of *M. sylvestris* contains polyphenol and flavone. This findings will start a new way for the development of functional food additives. Anthocyanin of *M. sylvestris* can be applied as a nature food additive and an ideal functional formulation because of its pigment property and bacteriostasic activity, which will give the bacteria a broad developmental prospect in such fields as food, health production and pharmacy, etc.

Conclusions

The bacteriostasic activity of anthocyanin to *Staphylococcus aureus* varied with increasing content of anthocyanin of *M. sylvestris*. In this study, 30 $g \cdot L^{-1}$ of anthocyanin of *M. sylvestris* had the most favorable bacteriostasic activity. And, this bacteriostasic activity could keep long time as anthocyanin was of the high concentration. Furthermore, when the content of anthocyanin of *M. sylvestris* was 10 $g \cdot L^{-1}$, the diameter of bacteriostasic circle was 6 mm on solid culture medium. With the content increasing up to 20 $g \cdot L^{-1}$and 30 $g \cdot L^{-1}$, the diameters of bacteriostasic circle were up to 13.5 mm and 16.0 mm, respectively. In addition, anthocyanin of *M. sylvestris* showed no obvious inhibition activity to *Escherichia coli* because *Escherichia coli* showed the nature-growing trend in liquid culture medium with different content of anthocyanin, as well as to *Aspergillus niger* because of no bacteriostasic circle formed on solid culture medium.

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